DETERMINATION OF THE WATER CONTENT OF COFFEE LEAVES USING INFRARED SPECTROSCOPY

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOLOGICAL ENGINEERING

DECEMBER 2016

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Contents

List of figures

List of tables

Abstract

Infrared (IR) spectroscopy and flat plate capacitors were examined as potential methods for the determination of leaf water content as alternatives to the current pressure bomb method. Flat plate capacitors were found to be a poor solution. IR spectroscopy was found to provide a good estimate of the leaf water content when using broad spectrum spectroscopy with partial least squares regression fitting (R^2=0.95). Normalized indices comparing reflectance between 1080 nm and 1200 nm ($R_{1080}R_{1200}$) and between 1250 nm and 1450 nm ($R_{1250}R_{1450}$) were found to provide strong correlations (R^2=0.90) with the commonly measured equivalent water thickness (EWT) and to provide reasonable (R^2= 0.70) correlation with the leaf water pressure as measured by pressure bomb.

Introduction

Coffee plants have highly irregular ripening patterns and must be water stressed to synchronize fruit ripening time. Mes (1957) demonstrated that periods of drought followed by reapplication of water can induce flowering in coffee. Further studies (A.C. Magalhães, 1976; Alvim Pde, 1960) produced evidence supporting the hypothesis that coffee trees must be first exposed to water stress before being irrigated to stimulate floral bud formation. Failure to apply this process of drought followed by application of water results in highly asynchronous ripening of coffee fruits (Drinnan, 1994) and thus an extended harvesting season. As a result, a great deal of research has gone into determining the ideal level of drought stress required for synchronizing coffee ripening. The role of water stress in coffee flowering is reviewed more extensively by Carr (2001).

More recently, papers have focused on how much water stress is necessary to release the flowers from their dormant state successfully. These studies have often focused on measuring leaf water pressure by pressure bomb (Scholander et al., 1965) due to the relative ease of use, as well as shorter time required than by oven drying the leaves. Studies by Turner and Long (1980) as well as by Meron *et al.* (1987) have related the water pressure to a more reliable thermocouple and psychrometer method. These studies found that the pressure bomb method is reliable when conducted properly (standard error of 2 bar compared to thermocouple psychrometer method), but may extend as far as 7 bar under certain conditions, such as poor wrapping of the leaf, and transporting for long periods of time after cutting. Meron *et al.* suggest using foil and plastic bag – the same technique adapted in present days with foil bags.

Reports of the level of water stress necessary to synchronize flowering time vary: Magalhães and Angelocci (1976) found that a leaf water potential of -12 bar was necessary, Crisosto et *al.* (1992) found -8 bar and Schuch et *al.* (1992) found that -26.5 bar was necessary. The negative pressures indicate that water would naturally flow into the leaf and that external pressure on the leaf is required for water to exit through the leaf's stem. Some of the discrepancy in these reports could be a result of the time of the day that measurements were taken. The water pressure of coffee leaves changes throughout the day (Kumar and Tieszen, 1980), so pre-dawn measurements are preferable to avoid diurnal variation in leaf water pressure. Crisosto et al. (1992) used predawn measurements, while Schuch waited until after the onset of photosynthesis, and Magalhães and Angelocci did not specify when measurements were taken. Another possible reason for the large variation can be found in the results of Meron et al. (1987), who found errors of between 2 and 7 bar may be present, when compared to the thermocouple psychrometer method. While the studies referenced likely kept variations as low as possible, even minor deviations in methodology may result in wide variations.

The pressure bomb method has several problems that leave room for potential improvement. The first is the destructive nature of the measurement; as the harm it inflicts on the plant makes frequent measurements or multiple simultaneous measurements from being practical. The second area for potential improvement is the equipment costs. A simple, low cost, hand pump up chamber costs $1,500; chambers that can be attached to compressed air tanks are more expensive. With either technology, this is a labor intensive process that requires a person to physically patrol the coffee fields and take samples. The process either requires a labor intensive pumping of the chamber (with as many as 30 or 40 pumps necessary to determine the water pressure), or the additional weight of an external air tank. In addition, when applied to coffee, the process requires pre-dawn measurements, because leaf water content changes throughout the day (Kumar and Tieszen, 1980). As a result, the process must also be conducted in the dark, making proper readings of the pressure chamber cumbersome. The high pressures involved in this process also pose a safety risk, as chambers must be able to withstand 20 to 30 bar or greater. While the pressure bomb method has been the preferred method for field testing of water stress for a long time, it seems that there must be a way to make these measurements without destroying plants, using high pressure, or possibly without using expensive equipment.

Research (Ghulam et al., 2008; Graeff and Claupein, 2007; Li et al., 2008; Liu et al., 2015) has shown that light absorbance in the infrared spectrum is related to the leaf water content. Water has a strong absorbtance in the IR spectrum at 1450 nm, 1940 nm, and 2500 nm, as well as weaker absorption peaks at 970 nm and 1200 nm. Infrared spectrometry has therefore been heavily researched in regards to determining water content in plants. Measuring infrared absorption or reflection in the region up to the 1650 nm portion of the spectrum is inexpensive. Using light instead of physical means for determining water content also solves the problem of destroying leaves with each measurement, thus allowing for many measurements of a given plant with no harm.

Based on his research Datt (1999) has suggested two measurement indices to determine the water content of Eucalyptus leaves based on leaf reflectance $(R_{850}-R_{2218})/(R_{850}-R_{1928})$ and $(R_{850}-R_{1788})/(R_{850}-R_{1928})$. The spectrum is generally clear of interference at around 850 nm, and is thus useful as a point of reference, allowing for comparison against other wavelengths, while removing the need to examine the overall leaf thickness.

Baret and Fourty (1997) have conducted other studies proposing the use of an inverted PROSPECT model (Jacquemoud and Baret, 1990), suggesting that there is indeed a relationship between leaf water content and a leaf's infrared properties. The overall work contributed by these studies has formed the basis for a large model basis to predict the spectral properties of various plants based on the parameters of chlorophyll content, dry mass content, and water content. The model forms a frequently used basis for theoretical experiments (Baret and Fourty, 1997; Li et al., 2008), many of which have been shown to correlate to real observations.

Some studies have attempted to expand on the known levels of IR absorption by water to implement large scale (field wide) monitoring systems using thermal infrared. These systems (Alchanatis et al., 2010; O'Shaughnessy et al., 2011; Pou et al., 2014) often times incorporate wet and dry reference panels, and incorporate ground based temperature readings. In the laboratory, these studies will often lay a leaf against a large thermal sink, for example laying the sample in water (Lopez et al., 2012). This method does not translate as well to the objectives of finding coffee water stress. There is furthermore, the issue that, as some researchers noted (Li et al., 2007), the amount of noise in the 1900 nm range of light practically eliminates the higher spectra from usability.

The relationships studied using large scale canopy level monitoring of plant water stress, such as those conducted on wheat (Li et al., 2007), are often times lower in overall correlation coefficient than studies conducted on individual leaves. Since coffee needs to be stressed to a level that is near the level that will cause significant defoliation, high margins of error in coffee monitoring are unacceptable because the levels of water stress are close to the levels that will kill or defoliate a plant. In contrast the field-wide monitoring, the monitoring of individual leaves allows for a greater level of confidence since measurements may be easily repeated.

Many of the studies into IR spectra have used slightly different portions of the IR spectrum in the generation of their correlations, while still forming strong relationships. This is to be expected, and indeed, a series of studies (Mobasheri and Fatemi, 2013; Wang et al., 2011;

Zhang et al., 2012) that have shown that there are large spaces with high correlation between leaf spectra and leaf equivalent water thickness (EWT). These experiments explored all possible 2 wavelength combinations at 1 nm increments using simple difference, simple ratio and normalized ratio methods.

While the application of IR spectrometry to the determination of EWT in plants is common, there are no studies relating IR spectra to leaf EWT in coffee. As can be seen from some of the studies already conducted, there is no single correlation relating water content and IR spectra for all plants, nor does any one set of wavelengths yield a high correlation between water content and IR spectra for all plants. If IR spectroscopy is to be applied to water level monitoring in coffee, the proper relationships must be evaluated. In addition to formulating proper indices, relationships must also be built that will relate to water pressure to the new indices, instead of relating them to equivalent water thickness (EWT), which is typically used. The relationship between leaf EWT and water pressure is not necessarily linear, as leaf water pressure incorporates variables such as the salt content of the leaf (i.e. osmotic pressure).

The amount of research into leaf water relations in other plants gives a high level of confidence that similar relationships can be determined for coffee. While direct comparison of EWT and water pressure are not easily available in the literature, the determination of the relationship is a simple matter, as the instruments for both measurements are easily available. If a correlation similar to the correlations in other plants can be found between the IR absorption of coffee leaves and the leaf water potential, then there is potential for a small piece of equipment to be carried into the field and used without the destructive process. Such a system would be much safer than current high pressure methods. Such a unit, being non-destructive to the plant, would not need to necessarily be as accurate as current methods, since the ability to measure multiple leafs from the same plant (or make multiple measurements of the same leaf) allow for greater confidence in measurements. If properly designed, it should also be much cheaper per unit than the current methods, while also reducing the preparatory labor involved. For example, LEDs are available in the infrared spectra corresponding to many of the peaks found in water for approximately $20 each. Broad spectrum photodiodes that measure the full spectrum at a reasonably flat response are also cheaply available (less than $5 for a sufficiently broad range photodiode or $20 for higher quality photodiode); these components, along with housing would constitute the bulk of the construction costs of a hand held unit.

An additional method that has been employed, but seen relatively little discussion in the literature, is the use of leaf's capacitive properties as a dielectric. Afzal et al. (Afzal et al., 2010) have found a relationship between the dielectric properties of a leaf and the leaf's water content. Here, the capacitive properties of various leaves were measured. Again, the study focused on a relationship between dry weight/wet weight ratio and the capacitance of a simple circuit using the leaf as a dielectric. If this correlation can also be found in coffee, it would provide for a potentially cheap, and independent means of confirming a leaf's water content. There are difficulties in the application of this technology, however, to coffee leafs, as well as to the use in the field. First, and foremost in these difficulties is the problem of the change of capacitance over the surface of the leaf. As was observed by Afzal *et al.*, the capacitive plates must be placed carefully on the leaf, as large veins in the leaf may change the measured capacitance dramatically. The study used relatively large capacitive plates. The larger plates, in turn, allow for a greater degree of averaging of the differences in capacitance along the leaf. They also have less to worry about in terms of veins. Coffee leafs have large veins branching out from the main center leaf, increasing the probability that one such vein will be caught in the capacitance measurement. The smaller leaf size also prevents large plates from being used in the process of measuring the plant.

The study also utilized higher frequencies than are commonly available on cheap capacitance meters (100 kHz and 1 MHz). There are a couple of reasons that this is noteworthy for the system. The first is that the capacitance of any material will change with the frequency of current applied to it. The second relates to their findings about the time required for water to polarize. The problem here lies in the presence of ions within the water. They found, predictably that the higher frequencies polarize the water more quickly; the capacitance reading changes while the ions in solution align with the electric field.

Despite the shortcomings of this method as they apply to coffee, it is worth examining this method and determining its suitability for measuring water stress in coffee. The primary reason it is worth attempting to adapt the strategy to coffee is that the study produced high correlation coefficients, indicating a high confidence level. An additional reason for searching for a correlation between capacitance and water content is that the measurement of capacitance is independent of any of the optical properties of a leaf, leaving errors in capacitance measurement as independent of those in spectroscopy.

Partial Least Squares

Partial least squares regression (PLS) is a matrix based method for fitting a y response variable to a large number of predictor variables (x variables) in a linear fashion, according to Equation 1.

$$\boldsymbol{Y} = \boldsymbol{XB} + \varepsilon \qquad (1)$$

Where ***Y***, ***X***, ***B***, and ***ε*** are all matrices. The method is credited to Herman Wold through a pair of papers (Wold). The solution of the matrix is achieved by first extracting a number of principle components (Mevik and Wehrens, 2007). The components are used in calculating the **B** matrix. As the number of principle components increases, so does the accuracy of the model. Incorporation of too many components into the model, however will lead to overfitting. The result of over fitting a model is that the initial data set fits very well, or even perfectly, but is a very poor predictor of future results. One common application of the PLS method is in modeling based on spectroscopic information.

The same features of the process that make it ideally suited for application in analytical chemistry are the same features that make it well suited to determining the water content of plants. While PLS has been used in the determination of water content in plants (Li et al., 2008; Li et al., 2007), PLS is not usually used. Instead, practice has tended toward the use of normalized indices between two wavelengths.

Materials and Methods

Pressure bomb measurements

A pump up pressure bomb chamber from PMS Instrument Company was used for pressure bomb measurements. Measurements were conducted according to manufacturer instructions (PMS Instrument Company, 2009). The leaf was placed in the pressure chamber with the stem sticking through the observation hole. The stem was freshly cut before pressurizing the chamber. The chamber pressurized at a rate of about 0.5 bar per pump, with 0.5 bar graduations on the pressure gauge. The chamber pressure where water began to form a meniscus on the protruding leaf stem was recorded. The leaf stem was observed after each pump under a 10x loupe.

Spectral reflectance measurements

The reflectance spectrum of each leaf was collected using an ASD Agrispec (ASD Inc. Boulder, CO). The spectrometer collects spectral reflectance at wavelengths of 350 nm to 2500 nm (3 detectors: 350-1050 nm; 1000-1800 nm; and 1800-2500 nm), with a 16 bit analog to digital converter with 1 nm of spectral resolution. Each leaf was sampled in 5 different locations, measuring the reflectance with 5 consecutive scans at each location with a white reference backing, and a small weight to ensure that the leaf was flat at the time of sampling. Five locations were scanned on each leaf yielding 25 scans per leaf. The scans were averaged together as a single spectrum for each leaf, with each point in the averages spectrum being the average of the 25 scans.

The standard deviation of each point's average was also calculated. The absolute standard deviations were divided by the total reflectance at each point. The average of these deviations was less than 5% of the average reading.

Two methods were used to analyze the IR spectra. The first was to compare two wavelengths according to Equation 2. In the second, two peaks were compared by normalizing the result according to Equation 3. The results of these comparisons were plotted, and linear regressions were performed. For comparisons with EWT, a quadratic equation and an exponential equation was also fit due to the high level of curvature present in the data.

$$R_{Wave1}R_{wave2} = \frac{R_{wave1}-R_{wave2}}{R_{wave1}+R_{wave2}} \tag{2}$$

$$R_{Peak1/Peak2} = \frac{R_{peak1/trough1}-R_{peak2/trough2}}{R_{peak1/trough1}+R_{peak2/trough2}} \tag{3}$$

Capacitance measurements

Leaf capacitance was measured in five locations on measured leaves. The capacitance was measured using a BK Precision 890C. Each leaf was placed between two tinned copper plates that were spring loaded to ensure constant pressure (60 Pa), as well as ensuring that the leaf was flat to the capacitor plates. In place of epoxy coating, a piece of paper was placed between the leaf and the top plate to prevent electrical contact. This system prevented problems with creating a thin but uneven surface of epoxy or other item between the capacitor plates.

Since the paper has a capacitance, it must be accounted for. While parasitic capacitances in the system can be zeroed out, the paper represents a capacitance in parallel with the plate. To compensate for this, parasitic capacitance was zeroed out from the system by opening the device 6 cm in the air to effectively leave the capacitance between the plates at zero. The capacitance of the paper was then measured, and subtracted out in calculations of parallel operation. The total capacitance measured was thus:

$$\frac{1}{C_{total}} = \frac{1}{C_{paper}} + \frac{1}{C_{Leaf}} \quad (4)$$

Leaf preparation

Prior to data collection, coffee plants (*Coffae arabica* typica variety) were kept at Magoon research center under a 50% shade Aluminet. During data collection, a polyethylene rain cover (Husky) was used to prevent rain from watering the plants. Sample leaves were chosen randomly from each plant. Each leaf was then cleaned with a dry paper towel and a metallic plastic bag was placed over the leaf for between 20 and 30 minutes to allow the leaf's water to reach equilibrium. The leaf was then cut off the plant with a sharp razor blade, as per the operator's instructions (PMS Instrument Company, 2009). The samples were taken to the lab for immediate measurements; starting with leaf pressure, then weight, followed by capacitance, then IR reflectance measurement.

Equivalent Water thickness measurements

The leaf fresh weight was recorded using a digital balance, before being dried under infrared heating lamp with 40% relative humidity until no weight change was observed when measured on a balance. The weight of the dried leaf was then recorded. The difference in weight was taken as the amount of water in the leaf. The volume of water, assuming a density of 1000 kg m^{-3}, was then divided by leaf area to yield the (EWT) of the leaf.

Leaf area measurements

Leaf area was determined by using the leafArea package in R (Katabuchi, 2015). Fresh leafs were scanned against a white background at a resolution of 600 DPI and saved in 32 bit bitmap format. The main function of leafArea (Run.ij) was invoked with parameters not related to pixels to distance remaining the same as defaults. The result was a leaf area in cm^2. Prior to use in this experiment, the software and scanner were checked by scanning sections of leaf cut to a known area. The tests included taking a known sized square (from previous scan) and cutting it into many pieces that were presented in different configurations.

Partial least squares

The PLS package (Mevik and Wehrens, 2007) for R was used for PLS analysis. The PLS analysis covered 10 components and compared the leaf water pressure, or the EWT, as Y variables with the IR spectrum from 675 nm to 2250 nm. The limited IR spectrum from 675-2250 nm was chosen to remove the noise from the beginning (350-675 nm) and end of the IR spectrum (2250-2500 nm) results. The kernel method was used for analysis. An additional analysis was conducted that combined the reflectance and the reflectance squared.

Generation of two data sets

When a plot was made comparing the EWT to leaf water pressure, there were many obvious outlier points. A q-q plot showed these errors to not be normally distributed. In order to improve the chances at obtaining a good relationship between IR indices and water pressure, the data was analyzed as two sets: the “edited” data set that removed points outside of the 99% confidence interval, and the original ”unedited” data set. Figure 1 shows the original plot without removal of data points and the 99% confidence interval.

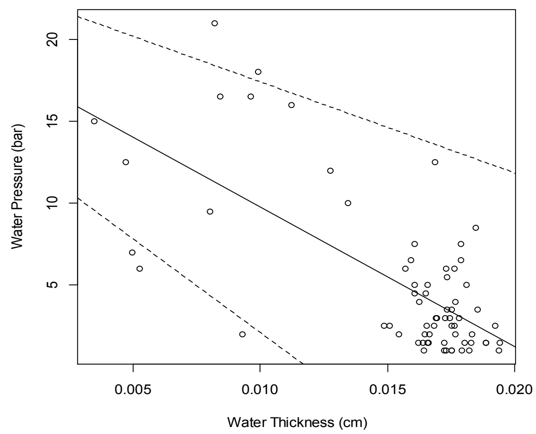


Figure 1. EWT vs. Water pressure plot with 99% confidence interval lines. The 5 points laying outside of the dashed lines in the figure were removed for the creation of the “edited” data set.

LED Evaluation

In order to determine the feasibility of adapting these findings to a LED/photodiode system, LEDs in the IR spectrum were selected from the Digikey website with wavelengths similar to the peaks found in these studies. In addition, due to the low cost, an LED with a peak of 940nm was also selected. The LEDs found are summarized below in Table 1. The most relevant parameters are the peak wavelength and the spectral bandwidth ($\Delta\lambda_{0.5}$). The spectral bandwidth corresponds to the difference in wavelengths for which the LED intensity is at 50% maximum value. Based on available datasheets for other LEDs, the LED intensity is negligible

Table 1. The LEDs found on Digikey.com that most closely match the peaks and troughs examined in this paper.

Peak (nm)	**$\Delta\lambda_{0.5}$ (nm)**	**Digikey Part #**	**Digikey price@ qty of 1**
940	90	1080-1350-1-ND	$0.55
1050	80	1125-1342-ND	$18.07
1200	80	1125-1335-ND	$20.42
1300	90	1125-1346-ND	$20.42
1450	90	1125-1354-ND	$20.42
1650	130	1125-1354-ND	$20.42

at the peak ± 2 $\Delta\lambda_{0.5}$.

While LEDs are available in 1450nm and 1650nm, and 1200nm wavelengths, these were the only values for which there is an available LED through Digikey. There are others available through some manufacturers such as Thorlabs (1080 nm for instance), but keeping a single source for a complete circuit board was explored first. Since no LED was available in the 1250 nm peak, both the 1200 nm and 1300 nm LEDs were evaluated. No sources were found for 1950 nm or equivalent.

Since the data sheets for these LEDs do not contain a spectral output plot, the forms were assumed to be parabolas centered on the peak wavelength and zeros at the peak+$\Delta\lambda_{0.5}$, and peak-$\Delta\lambda_{0.5}$. The parabolic form of the LED spectra would tend to overestimate the contribution of portions that are not part of the spectrum of interest. This form was assumed when multiplying by the leaf spectrum to assess the response that would be expected with each of the individual LEDs. Response to the LED for each leaf was predicted by numerically integrating (rectangle rule) the product of the presumed LED intensity curve by the recorded leaf spectra and assuming a flat photodiode response. The normalized responses were calculated and a linear fit to the water pressure was estimated for each possible combination of two LEDs.

Results and Discussion

Water pressure and equivalent water thickness

Figure 2a plots the water pressure as measured by the pressure bomb method against the equivalent water thickness. The relationship follows a linear correlation, however, the R^2 value is very poor ($R^2 = 0.47$), indicating a large amount of measurement variation. When a small number of points are removed (the points outside of the 99% confidence interval of this fit), the

R^2 improves to 0.70 (Figure 2b). Examination of the residuals from this fit shows a standard deviation of 2.6 bars (0.26 MPa). Since there is a possibility that a correlation between EWT and water pressure is exponential in nature, the values of water pressure were correlated linearly against the natural log of EWT (i.e. the water pressure was plotted against ln(EWT)). The results showed that the data without removal of points had reduced correlation (R^2=0.40), but the correlation improved to an R^2=0.72 when data points were removed, indicating that any relationship between leaf water pressure and EWT may be logarithmic.

When searching for the reason for this poor correlation, it is tempting to look first at the differences in the measurement accuracies. The measurements for EWT are straight forward, and rely on verified technologies with small errors (i.e. balance with 1 microgram of resolution and scanner that can scan to 0.004 cm resolution) compared to the overall measurements. The pressure bomb method, meanwhile relies on the human eye to determine at what point the water meniscus forms. In addition to a 0.5 bar resolution over 20 bar maximum pressure before accounting for the possibility of at most 0.5 bar over pressurization (1 pump of the chamber yields 0.5 bar pressurization).

One of the first places to look in exploring why these two values are not correlated as tightly as expected is in the potential sources of error for each quantity. There are relatively few sources of error in the measurement of EWT. The scanner used has 600 dpi resolution (236 dots per cm), which, while low by current standards, means that a single pixel is 1.8 E-5 cm^2, which, given the size of the average leaf (approximately 36 cm^2 with a perimeter of approximately 33 cm) which results in a total error of 800 parts per million (ppm). The measurements of the weights are accurate to within 1 mg over approximately 1 gram (1000 ppm). The propagation of errors analysis shows that this is expected to be accurate to within 60 ppm. The results shown later in the study show that the EWT measurement is highly correlated with the IR spectrum. Meanwhile, the water pressure method relies on human judgement, and the human eye to determine when the water meniscus has formed. It also relies on the accuracy of the instrument, which has a resolution of 0.5 bar, compared to a maximum of 20 bar. The error for the purposes of this experiment may represent between 2.5% of the total value to as much as 33% (2,500 ppm -33,000 ppm) of the measured value.

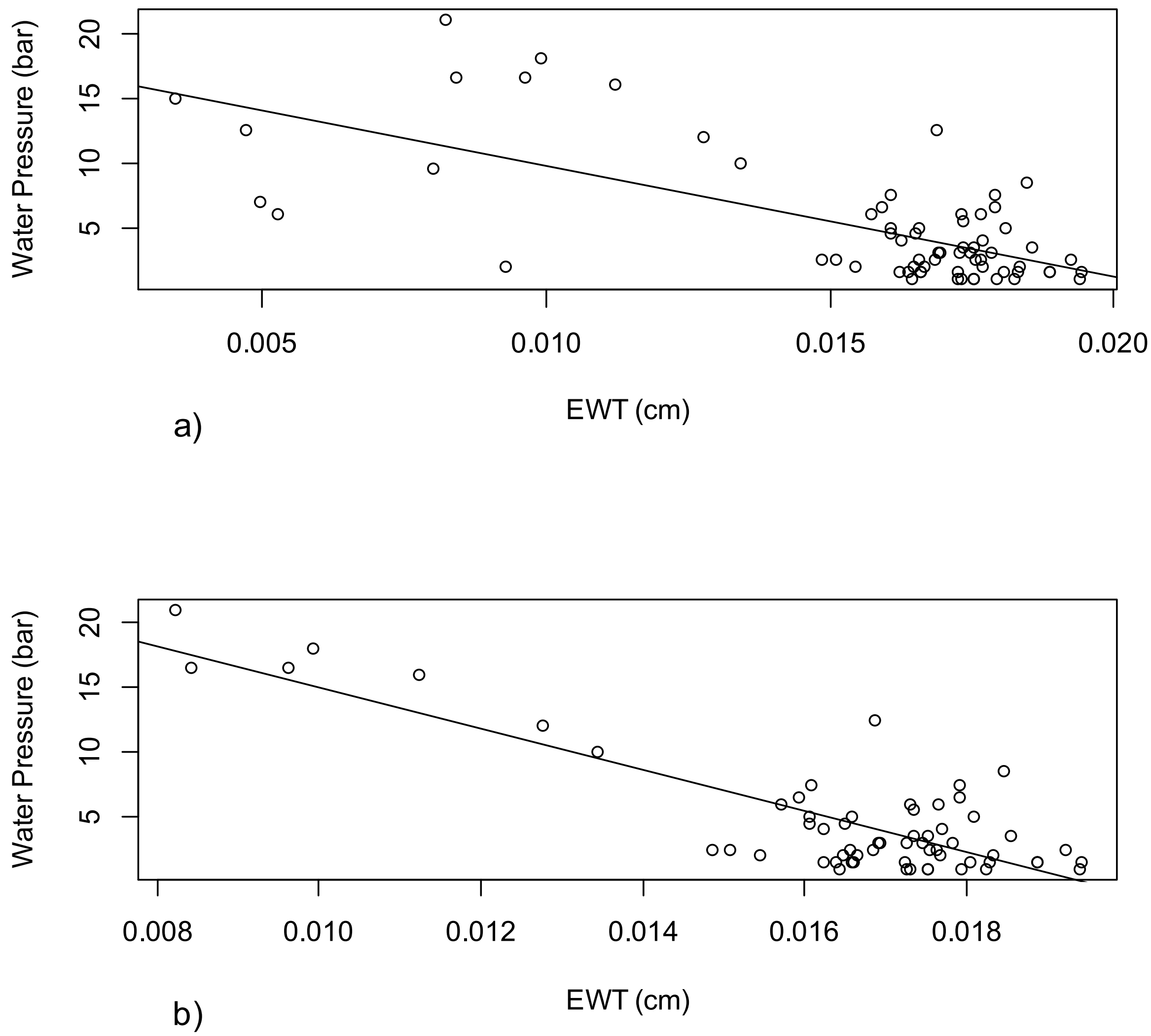


Figure 2 comparison of EWT and water pressure for two data sets: a) unedited and b) edited.

Table 2 Pressure bomb repeatability results.

Leaf	Read 1 (bar)	Read 2 (bar)	Read 3 (bar)	Read 4 (bar)	Read 5 (bar)
1	3.5	4.0	3.5	4.0	3.5
2	4.5	4.5	5.0	4.5	5.0
3	3.5	3.5	3.0	3.5	3.0
4	5.0	5.5	5.5	5.5	5.5
5	4.5	4.5	5.0	4.0	4.5

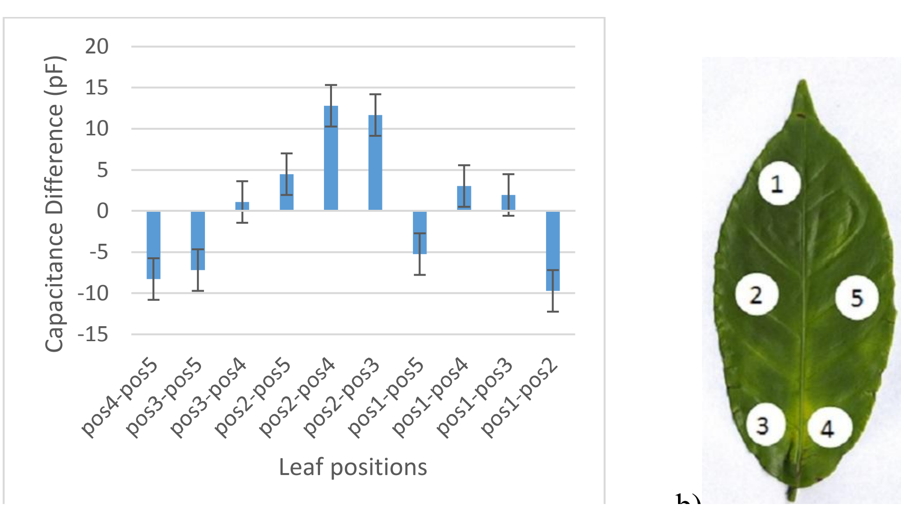
However, the pressure bomb method is well-established, with supporting evidence taken from other methods of measuring the water pressure. Boyer found this method to be accurate to within 2 bars using a thermocouple psychrometer; this was confirmed by Duniway (1971). Since there have not been studies showing the relationship between EWT and water pressure, it is not possible to directly compare with the literature to determine if the results found here are accurate. In order to determine the possibility of human error, another experiment was carried out to measure the leaf water pressure of several leafs multiple times in succession. The results, shown in Table 2, demonstrate that the pressure bomb method generates a very reliable reading, with variation of at most 1.0 bar over a wide range of water pressures. Given this data, human error as a systematic source of error can be dismissed.

Another possibility is that the water pressure of a leaf is less direct of a measurement than other measurements. For example the EWT is a direct measurement of the water present in the leaf, and the water pressure of the leaf will naturally vary with variables such as the osmotic pressure of tissue cells. For this reason, there would naturally be expected to be a slight difference in these two quantities. Since the removal of obvious outliers increases this correlation so much, it seems most likely that this is the primary cause of disparity between these two quantities, combined with some additional room for human error in measurements. Upon review of notes, it was observed that all but one of the points removed belong to leaves that had suffered from visible necrosis by the time they made it to the lab. The necrosis would lead to an increase in the water present outside of cells, resulting in a reduced leaf water pressure measurement.

The removal of points results in two sources of data for measurement: “edited” and “unedited”. As will be seen later in the paper, when correlating water pressure to IR, the edited data set forms stronger correlations (higher R^2) than the unedited data set. By contrast, when correlating EWT against IR, the unedited data set forms stronger correlations than the edited data set. This, combined with the fact that far more than 1% of the data falls outside of the 99% confidence interval of the fit (Figure 1) leads to the conclusion that there are some anomalies in the water pressure data; for a data set of 70 points, one would expect one or two points to fall outside of this interval, while in reality, 5 points fell outside of the window. A normal Q-Q plot of the data (not shown) also shows that the residuals are not normally distributed for the unedited data; the Q-Q plot shows a much more normal distribution of error for the edited data set. The generation of two data sets will be seen to allow for strong correlations with both water pressure to the edited data set, and to EWT for the unedited data set.

Capacitance

The capacitance of a leaf differed along the length of the leaf, rendering it difficult to accurately measure the capacitance. Other studies have shown correlations between the capacitance of leafs and the moisture content (Afzal et al., 2010). The results of this study showed no strong or noteworthy correlation between these quantities. There are two possible reasons for this. The first is that the instruments used were not sufficiently sensitive, or did not employ an effective frequency to estimate the water content of the leaves (studies typically utilize frequencies of 100 kHz or higher). Since the study aimed to determine the low cost relationships, a 1 kHz capacitance meter was used. The second possibility lies in the variation of



a) b)

Figure 3. a) capacitance differences between positions and b) the locations where capacitance was measured on each leaf

capacitance along individual leaves.

Above in Figure 3, the difference between capacitance measured at each position; Figure 3b shows where each of the capacitances was measured. The results show that the capacitance differs greatly from the middle of the leaf to each of the tips and the stem of the leaf. There is a smaller difference in measured capacitance (pF) between the tip and the stem.

Analysis of the deviations of the measurements at these positions shows that there is less deviation at the tip and stem of the leaf than in the center, making one of these locations a more appropriate target for measuring capacitance. Overall, the level of change from tip to stem of the capacitance makes for unpredictable results. While in the laboratory, it may be possible to control the system sufficiently to get reproducible results, the amount of training that would be required to get predictable results in the field indicate that even with correlations between capacitance and leaf water content, the capacitance is an unreliable field measurement method under the conditions of this study.

Because the flat plate capacitor used in this study is not a previously tested measurement device, there exists a possibility that the instrument will give false readings. This seems especially possible given the results from the main experiment. However, the standard error within the same location without moving the instrument indicates that the instrument and method are good for measuring capacitance in the system (i.e. the deviation from location to location is a function of the changes in leaf structure rather than randomness of the instrument). The fact that the instrument gives different readings at different locations within the leaf and within the same location on different leafs also supports the conclusion that the leaf structure is the primary culprit in making capacitance an unreliable field measurement.

Infrared Spectroscopy

A typical IR spectrometry result for coffee leafs is shown below in Figure 4. There are 6 peaks and 5 troughs that are of interest. The valleys of these peaks occur at the wavelengths of approximately 650, 970, 1200, 1450, and 1950 nm. These results agree with other literature (Behrens et al., 2007; Datt, 1999; Eitel et al., 2006; Seelig et al., 2008; Zhang et al., 2012) on the subject of leaf water response, and have been shown to respond to water content. The 650 nm valley likely belongs to chlorophyll (Conejo et al., 2010). The level of these troughs relative to their neighboring peaks has been shown in other literature to be highly correlated with the equivalent water thickness. Also of note is that the wavelength where the correlation coefficient for water pressure is in close agreement with the literature for other plants when the EWT is measured using the same methods (Mobasheri and Fatemi, 2013). The correlations in coffee are much stronger when

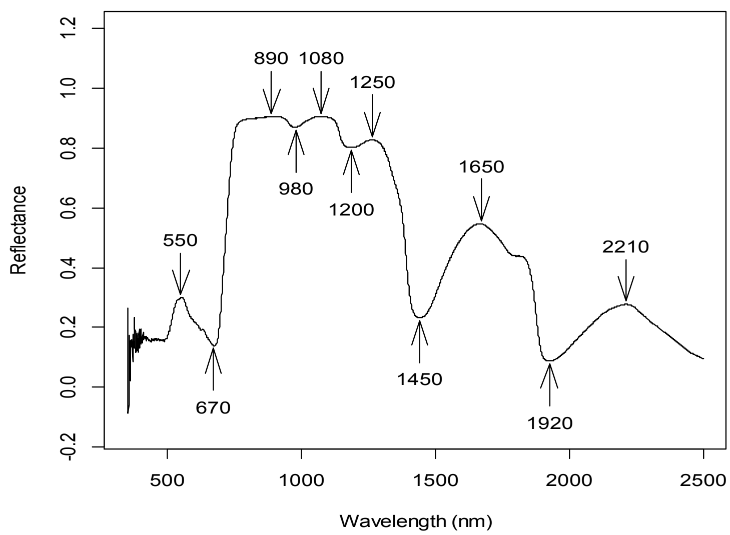


Figure 4. Typical IR reflectance spectrum. Peaks and troughs used in analysis labeled.

EWT is measured instead of water pressure. The results show that for coffee, the troughs at 1200 nm and 1450 nm are the ideal peaks to measure for determining water stress.

There are much stronger correlations with EWT than with water pressure, with only one peak achieving a reasonably strong R^2 value when measuring water pressure, and three peaks, plus combinations of these peaks, achieving a R^2 above 0.70. This is to be expected, as there are fewer potential sources for error in EWT measurements than with water pressure measurements. The strongest correlations for both EWT and leaf water pressure methods occur at the same wavelengths. It is worth noting that the spectral features at 1200 nm and 1450 nm both give relatively high correlations, indicating the region is a good candidate for LED/photodiode system development.

Table 3. Linear correlations for the relationship between water pressure and spectral peak reflectance's. Equations were fit to the form of WP=ax+b. Where WP is the water pressure in bar and x values are given by the reflectance according to Equation 2.

Unedited Water Pressure				**Edited Water Pressure**			
Index	***a***	***b***	***R^2***	***Index***	***a***	***b***	***R^2***
$R_{550}R_{650}$	17.8	-48.6	0.34	$R_{550}R_{650}$	34.4	-108	0.50
$R_{800}R_{970}$	4.96	-52.5	0.31	$R_{800}R_{970}$	6.62	-177	0.68
$R_{1080}R_{1200}$	16.9	-216	0.45	$R_{1080}R_{1200}$	32.3	-474	0.70
$R_{1250}R_{1450}$	24.6	-37.9	0.43	$R_{1250}R_{1450}$	47.1	-79.0	0.64
$R_{1650}R_{1950}$	34.2	-44.1	0.37	$R_{1650}R_{1950}$	48.2	-64.5	0.39
$R_{1080}R_{1200}/R_{1250}R_{1450}$	-56.7	-75.8	0.31	$R_{1080}R_{1200}/R_{1250}R_{1450}$	-202	-257	0.61
$R_{1080}R_{1200}/R_{1650}R_{1950}$	-65.7	-83.2	0.37	$R_{1080}R_{1200}/R_{1650}R_{1950}$	-177	-216	0.61
$R_{1250}R_{1450}/R_{1650}R_{1950}$	-1.92	-54.7	0.33	$R_{1250}R_{1450}/R_{1650}R_{1950}$	-13.6	-159	0.58

In searching for ideal spectral indices to determine the water content of a leaf, there are numerous possibilities for combining two wavelengths. The research by Mobasheri and Fatemi (Mobasheri and Fatemi, 2013), included a heat map of correlations when taking the difference, simple ratio, and normalized ratio between two or more wavelengths. Their findings suggest that the normalized ratio between two wavelengths (transformation of equation 2) is the ideal method for identifying water content. Zhang et al. (2012) have produced similar heat maps of R^2 value with similar results. These findings largely agree with their findings, with the exception of finding higher correlation strengths at 1450nm than they did, while having slightly weaker correlations at 1650 nm. Indeed, the literature is full of suggested and tested correlations. Table 3 shows the results of the normalized ratios found in this study, when fit to a line of y=ax+b. In order to determine if there is an exponential relationship between water pressure and IR spectra, a semi-log plot was constructed to explore the possibility that the relationship $y=ae^{bx}$ produces a correlation. The results of the semi-log method are shown in Table 4. The R^2 values for this method are all lower than for the linear fits against water pressure for the same index.

Table 4. Exponential correlations for the relationship between water pressure and spectral peak reflectance's. Equations were fit to the form of ln(WP)=ax+b. Where WP is the water pressure in bar and x values are given by the reflectance according to Equation 2. The resulting equation is thus $wp=ae^{bx}$.

Unedited Water Pressure				**Edited Water Pressure**			
Index	***a***	***b***	***R^2***	***Index***	***a***	***b***	***R^2***
$R_{550}R_{650}$	29.2	-8.15	0.29	$R_{550}R_{650}$	216	-15.3	0.31
$R_{800}R_{970}$	3.41	-8.89	0.27	$R_{800}R_{970}$	4.19	-24.3	0.39
$R_{1080}R_{1200}$	24.6	-35.8	0.38	$R_{1080}R_{1200}$	175	-68.5	0.45
$R_{1250}R_{1450}$	86.4	-6.23	0.36	$R_{1250}R_{1450}$	1.41×10^3	-11.3	0.40
$R_{1650}R_{1950}$	431	-7.31	0.32	$R_{1650}R_{1950}$	1.92×10^3	-9.48	0.26
$R_{1080}R_{1200}/R_{1250}R_{1450}$	9.67×10^{-5}	-12.9	0.28	$R_{1080}R_{1200}/R_{1250}R_{1450}$	6.31×10^{-13}	-36.4	0.38
$R_{1080}R_{1200}/R_{1650}R_{1950}$	2.72×10^{-5}	-13.8	0.31	$R_{1080}R_{1200}/R_{1650}R_{1950}$	2.80×10^{-11}	-30.3	0.37
$R_{1250}R_{1450}/R_{1650}R_{1950}$	1.10	-9.00	0.28	$R_{1250}R_{1450}/R_{1650}R_{1950}$	0.270	-21.5	0.33

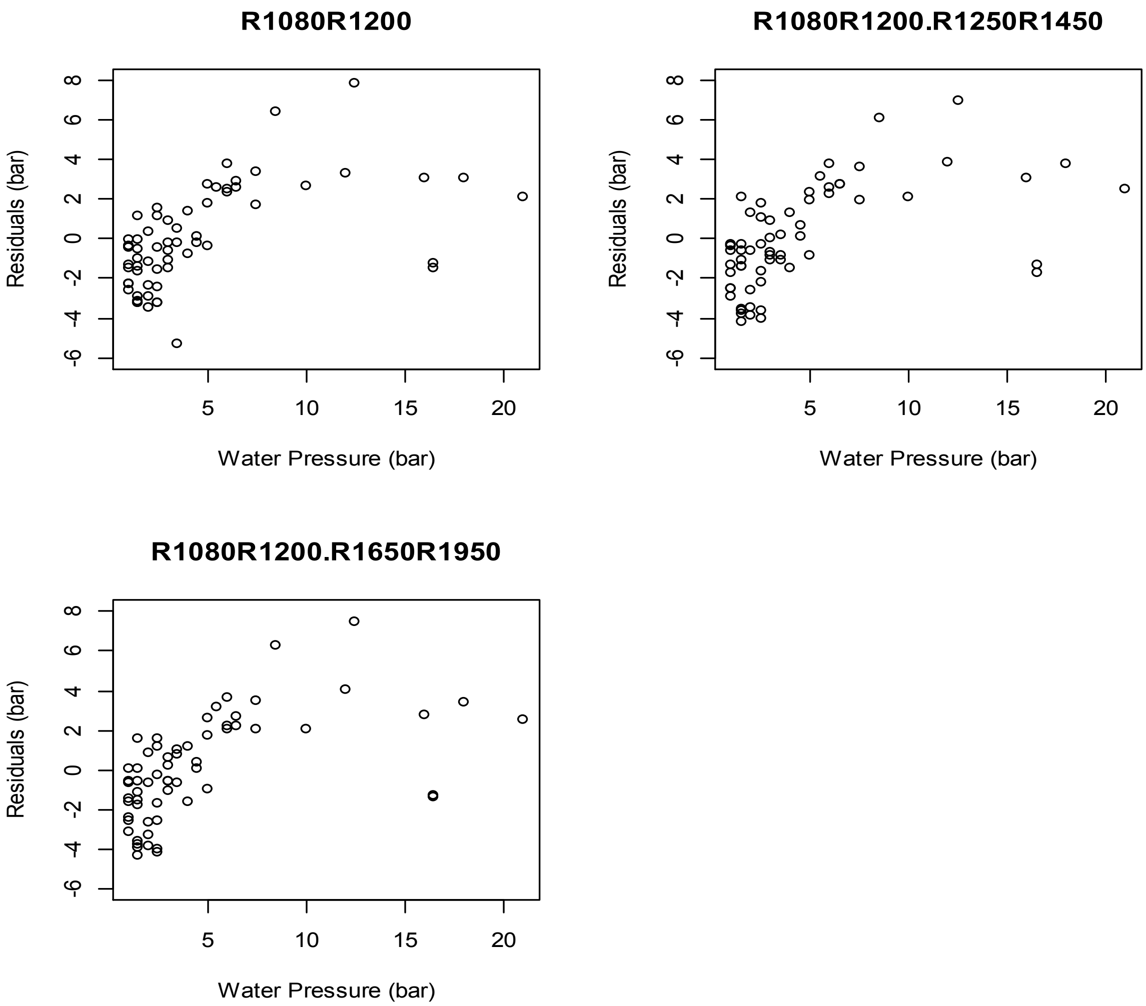


Figure 5. Residuals for water pressure relation when comparing between two peaks.

These results are consistent with other studies (Penuelas et al., 1997) that have found that the peak $R_{800}R_{970}$ is significant in several Mediterranean plants. In the literature (Wang et al., 2011), this peak is often found to be one of those most closely correlated with water content, or its reverse, dry mater content. Examining the results of other research (Mobasheri and Fatemi, 2013; Zhang et al., 2012) shows that the range of wavelengths for which the 800 nm region produces a strong correlation is very small, with very low correlations surrounding them. The other correlations found in this study approach those found in previous studies for other plants at the indices of $R_{1080}R_{1200}$ and $R_{1250}R_{1450}$. Figure 2 shows the linear correlation results of comparing the water pressure to individual peaks. Overall, the peaks method for comparing leaf water pressure with IR spectra produces poor results. The unedited data set shows no correlations of value (peak R^2 of 0.45), while the removal of data points as described above results in three peaks of useful correlations (R^2 >0.60). These indices are $R_{800}R_{970}$ (R^2=0.68), $R_{1080}R_{1200}$ (R^2=0.70), and $R_{1250}R_{1450}$ (R^2=0.64). In addition to these single wavelengths, there are two peak combinations that give strong correlation results; $R_{1080}R_{1200}/R_{1250}R_{1450}$ (R^2=0.61), and $R_{1080}R_{1200}/R_{1650}R_{1950}$ (R^2=0.61) (residuals shown in Figure 5). None of these combinations of peaks improve the correlation coefficients of the peaks involved, leaving normalized examination of the two wavelengths as the ideal method for determining these relationships. The peaks only reach a level of significance when the spectrum is read from lower wavelength to higher wavelength, although the peak at 1450 nm approaches significance in this context. Since previous studies examining water content and IR spectroscopy have focused on the EWT, rather than the leaf water pressure, direct comparison of this data with the literature is not possible. However, the peaks that have been found in this study compare well with data found in the case of EWT.

While some studies have found that the peak found at 650 nm ($R_{550}R_{650}$) is correlated with water content, it also corresponds to the IR spectrum of chlorophyll. Therefore, it is expected to be correlated to water content indirectly. Due to the fact that the peak is at beast an indirect measurement, as well as the ephemeral nature of this correlation even within this study, it can be disregarded for usefulness in a commercial device.

Given the relatively low quality of the correlation between water pressure and water content, it should not come as a surprise that the correlations between water pressure and IR are weaker than the literature when comparing EWT. It is encouraging, that the correlations found between the 1200 nm and the 1450 nm peaks are at least comparable to the findings of Seelig *et al.* (2009). These correlations also are similar in strength to some of the correlations found in the literature when EWT is used.

Previous studies have focused largely on only linear correlations between the reflectance at these wavelengths, and the EWT. Other studies have also found that the combination of peaks will improve the correlation. It is interesting to note that the use of only one or two peaks instead of the entire spectrum appears to produce better results than PLS method. It could, therefore be possible to improve results of PLS by using only small portions of the spectrum.

Since the purpose of this study is to determine the suitability of IR in designing a small handheld device that does not require precision equipment to indirectly measure the level of water stress coffee plants are experiencing, a brief evaluation of the spectra found to be significant is also worthwhile. The spectra found in this study suggest that there is a continuous portion of the IR spectra where a plant leaf will produce strong correlations. The continuous portion of significance allows for a single photodiode to evaluate a continuous spectrum. Meanwhile, the locations of the peaks found correspond well to commercially available LEDs.

In particular, it is easy to obtain LEDs that emit at or near the observed peaks and troughs with narrow spectrum (for example 1050 nm, 1450 nm, 1650 nm, and 1200 nm LEDs with a 100 nm range are available for $20 per each, and 980 nm for less than $1 in single quantity through Digikey). Since these are likely the most expensive component in a light based water stress device, this gives high potential for relatively low cost spectral solutions.

IR spectrum – EWT, peaks

Table 5. Relationships between EWT and normalized IR indices

	Unedited EWT=ax+b,			*Edited EWT =ax+b*		
Index	R^2	a	b	R^2	a	b
$R_{550}R_{650}$	0.75	2.8E-04	5.8E-02	0.51	7.8E-04	5.7E-02
$R_{800}R_{970}$	0.80	1.6E-02	6.8E-02	0.78	1.5E-02	9.9E-02
$R_{1080}R_{1200}$	0.89	2.1E-03	2.5E-01	0.87	3.9E-04	2.8E-01
$R_{1250}R_{1450}$	0.85	-6.6E-03	4.3E-02	0.73	-7.3E-03	4.4E-02
$R_{1650}R_{1950}$	0.68	-1.6E-02	4.8E-02	0.39	-6.2E-03	3.4E-02
$R_{1080}R_{1200}\backslash R_{1250}R_{1450}$	0.82	9.6E-02	9.9E-02	0.81	1.4E-01	1.5E-01
$R_{1080}R_{1200}\backslash R_{1650}R_{1950}$	0.85	1.0E-01	1.0E-01	0.82	1.3E-01	1.3E-01
$R_{1250}R_{1450}\backslash R_{1650}R_{1950}$	0.83	2.4E-02	7.0E-02	0.78	2.8E-02	9.6E-02

While the water pressure is the information most commonly used by people studying flowering in coffee, the EWT is most commonly studied by people using IR spectroscopy to determine water content. As a result, the comparisons to this particular study serve as a means to confirm that the results are in line with other results as well as with other plants. In contrast to the water pressure method, the EWT method shows correlations without the removal of data points, indicating that the points that were found to be outside of the 99% confidence interval in the analysis of EWT vs. water pressure were a result of improper technique in water pressure measurements. Similar to the comparison of peaks and water pressure, the peaks at 1200 nm and

1450 nm have a high level of correlation when fit in a linear plot. Normalizing a combination of two indices is another method that has been used by some researchers to attempt to improve the accuracy of their models. Combining indices does improve the correlation for many of the indices in the unedited data set, although it does not do so in the edited data set (Figure 7).

While these linear plots of unedited data (Figure 6, Figure 7, residuals in Figure 8, Figure 9) show a high correlation, they also show a slight curvature that is more evident when a linear curve is plotted with them. This curve largely disappears in the edited data (Figure 10, residuals in Figure 11). This curvature suggests that a power curve may be a better fit to the equation. To explore this curvature, two additional plots were generated for each data set. The results of these fits are shown in Table 6. Because analysis of the edited data set results in lower correlation coefficients, only the unedited data set results are included here.

Table 6. Relationships between Quadratic ($EWT=ax^2+bx+c$), and exponential ($EWT=ae^{bx}$ indices.

	$EWT=ae^{bx}$			$EWT=ax^2+bx+c$			
Index	R^2	a	b	R^2	c	b	a
$R_{550}R_{650}$	0.81	3.35E-03	5.71	0.75	1.9E-4	6.0E-2	-2.9E-3
$R_{800}R_{970}$	0.85	1.50E-02	6.62	0.86	1.5E-2	0.12	0.34
$R_{1080}R_{1200}$	0.91	4.09E-03	23.5	0.89	3.1E-3	0.17	0.95
$R_{1250}R_{1450}$	0.89	1.66E-03	4.14	0.85	-1.1E-3	1.5E-2	3.3E-2
$R_{1650}R_{1950}$	0.72	6.76E-04	4.68	0.68	-3.1E-3	3.0E-3	3.8E-2
$R_{1080}R_{1200}\backslash R_{1250}R_{1450}$	0.89	4.04E+01	9.71	0.85	0.43	0.87	0.45
$R_{1080}R_{1200}\backslash R_{1650}R_{1950}$	0.90	6.67E+01	9.92	0.86	0.28	0.50	0.22
$R_{1250}R_{1450}\backslash R_{1650}R_{1950}$	0.90	3.69E-02	6.88	0.85	3.0E-02	0.14	0.17

When examining the combined peaks, the correlation with EWT is reduced very slightly when examining the edited data set. Like with the water pressure method, this method also shows that the combination of two points improves the accuracy of the model over any of the single points. However, it also presents an economic tradeoff, as the accuracy improvement is only slight, while the costs of adding another peak could present a significant investment if LED/photodiode systems are used.

While the IR vs. EWT study agrees with previous studies (Mobasheri and Fatemi, 2013; Penuelas et al., 1997; Seelig et al., 2008; Wang et al., 2011; Zhang et al., 2012) in terms of R^2 values and ideal wavelengths for comparison, the results of fitting against exponential and quadratic equations is something that is not always explored. However, when measuring reflectance in the manner that it has been in this experiment (i.e. with a white reference background), the Beer-Lambert law predicts an exponential relationship between normalized IR indices and the EWT (Seelig et al., 2008).

The results also concur with another set of studies by Seelig (2009). These studies examined the wavelengths at 1300 and 1450 nm to measure the water content in beans, sugar beets, and snow peas. While the specific wavelengths differ slightly (1250 nm instead of 1300 nm) from those in the present study, the overall correlation strength remains high in both studies. This study reinforced that the method used by Seelig *et al.* is applicable to other plants. As noted in the water pressure section, other studies have shown strong R^2=0.95 in the region comparing 1300 nm to 1450 nm (Mobasheri and Fatemi, 2013), and also shows that there is a range of values surrounding each of the wavelengths that will produce significant correlations.

The overall results in comparing against EWT generates a high degree of confidence in the data set, allowing for further examination against water pressure. The regions of strong correlation are the same as the regions found by other studies in other plants. These studies also demonstrate that the relationship between EWT and water pressure is real, even if not as strong as desired by showing some of the same trends in spectral relationships (the rank of the strengths of the relationships are the same between the two studies).

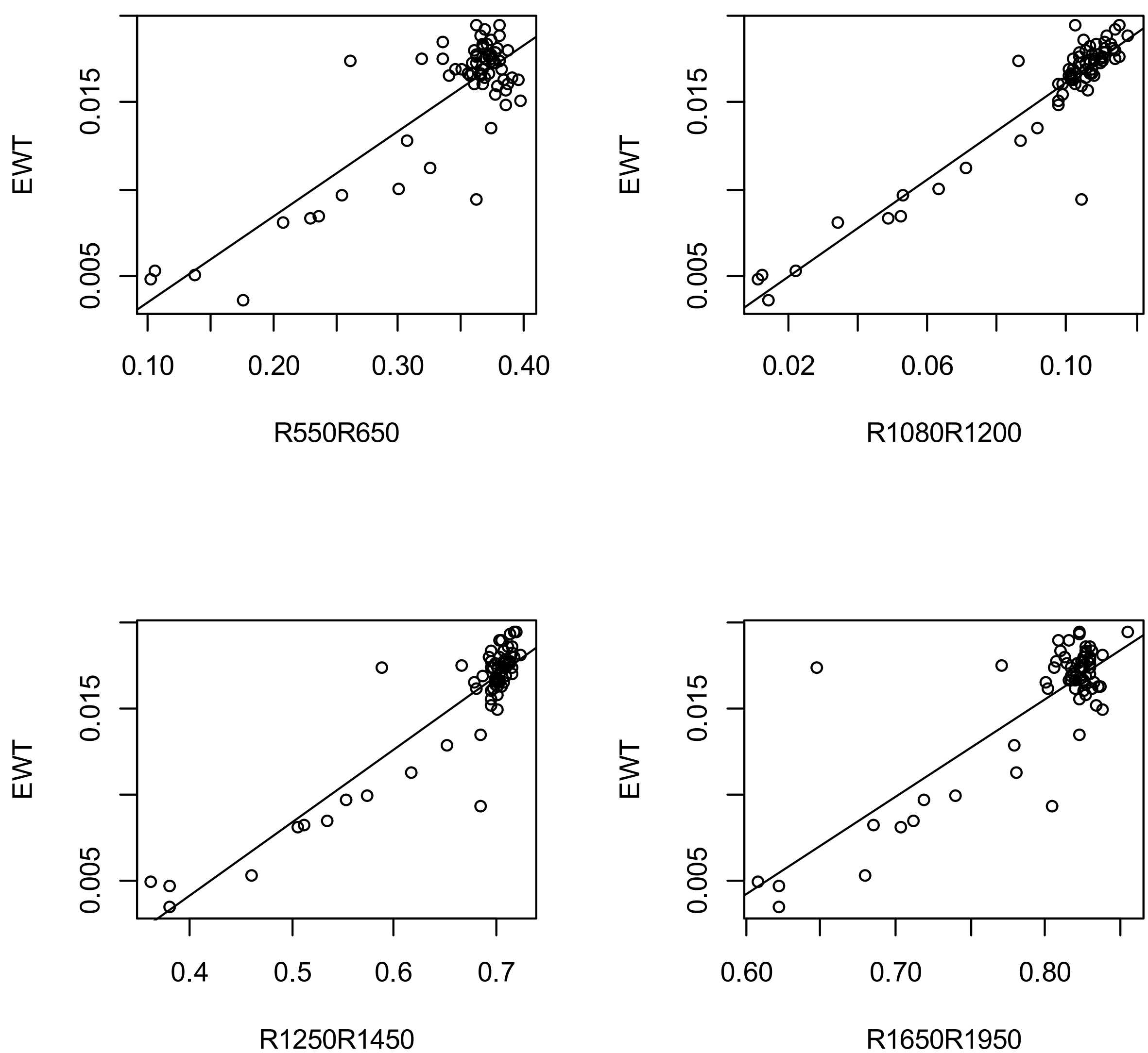


Figure 6. Plots of EWT vs. one normalized value.

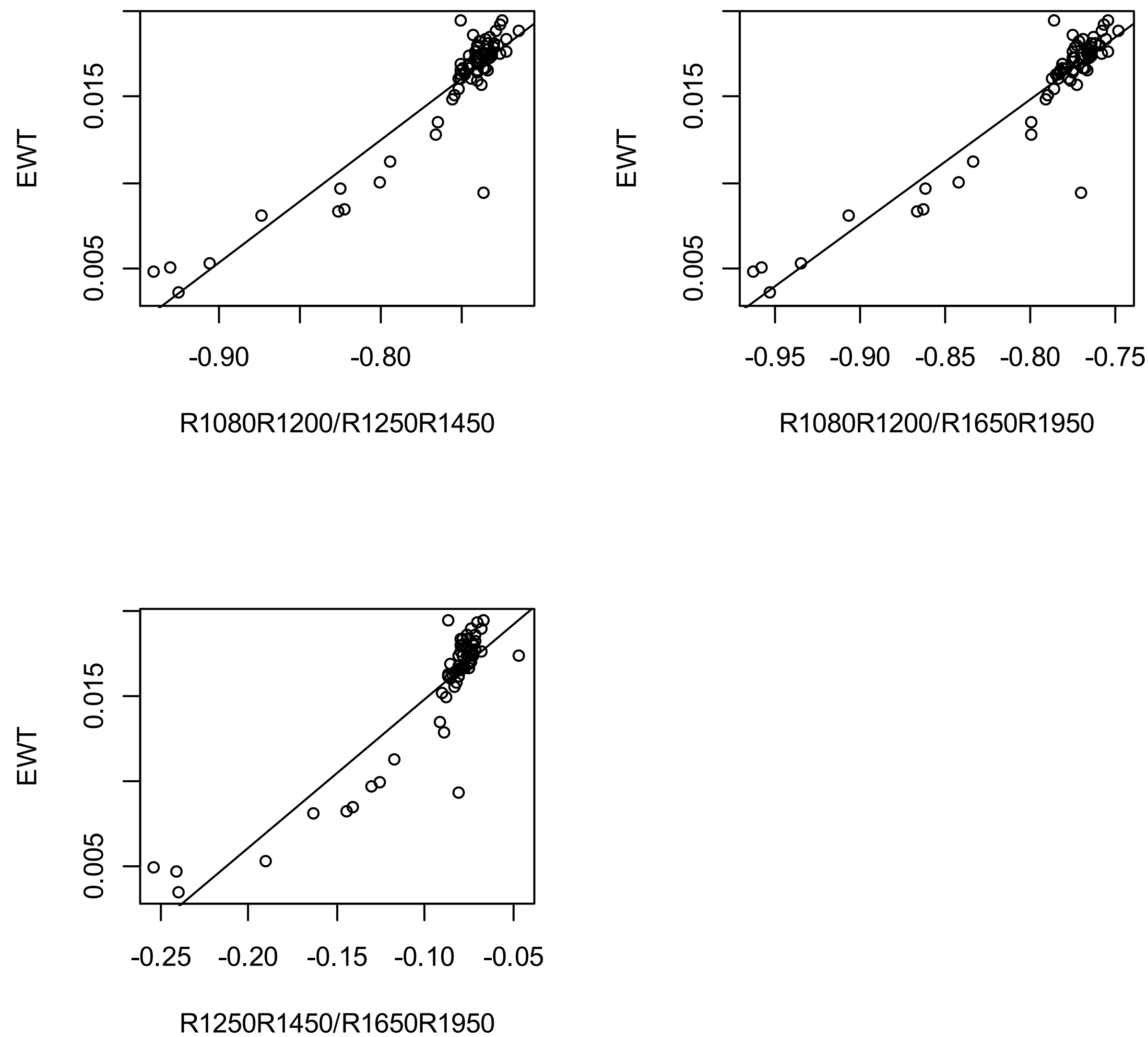


Figure 7. Plots comparing EWT to two normalized peaks.

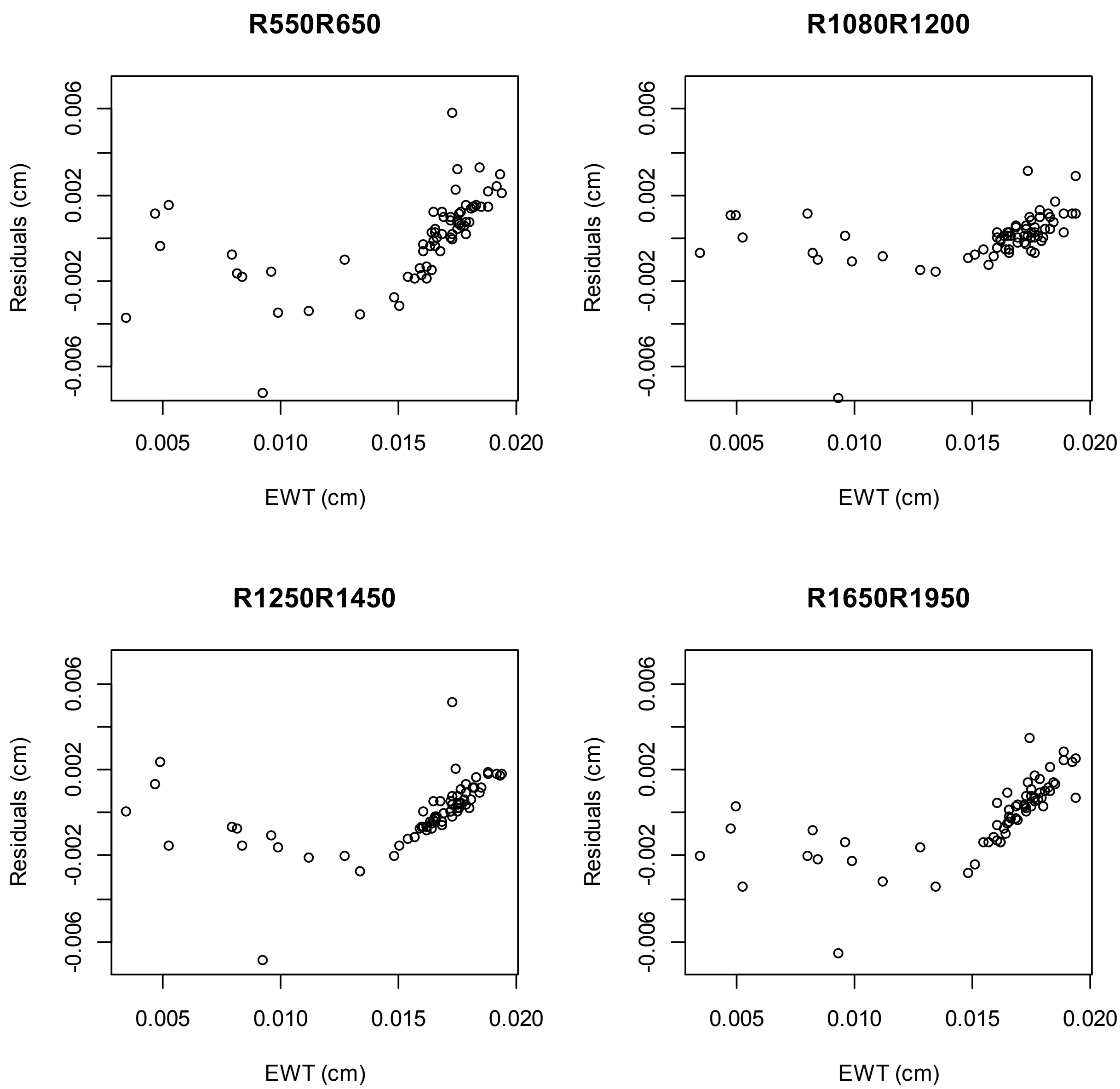


Figure 8. Residuals graphs for single peaks when comparing EWT. All points fall within 0.006 cm of measured values, and most fall within 0.002 cm.

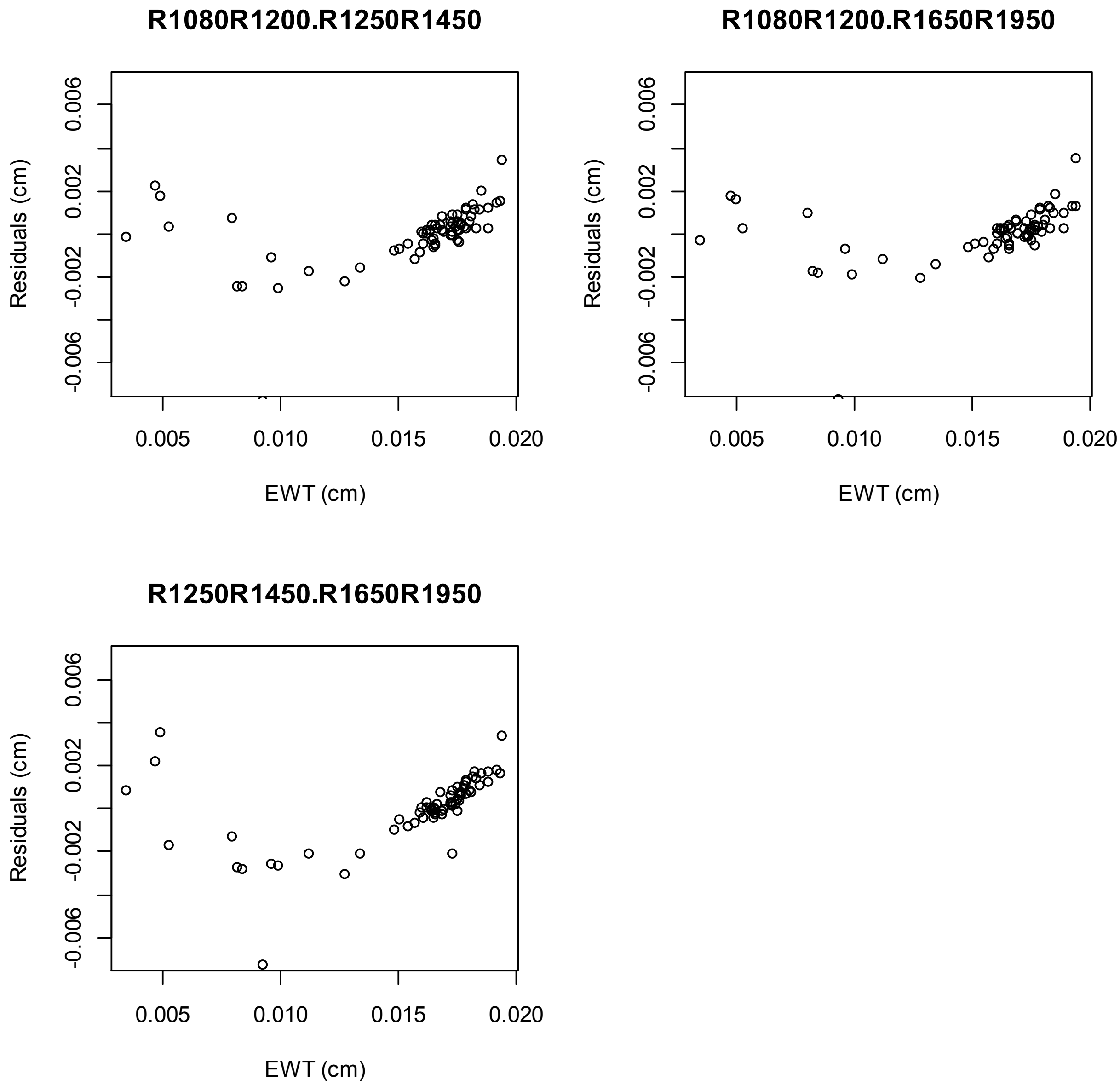


Figure 9. Residuals plots for relations combining 2 or more peaks.

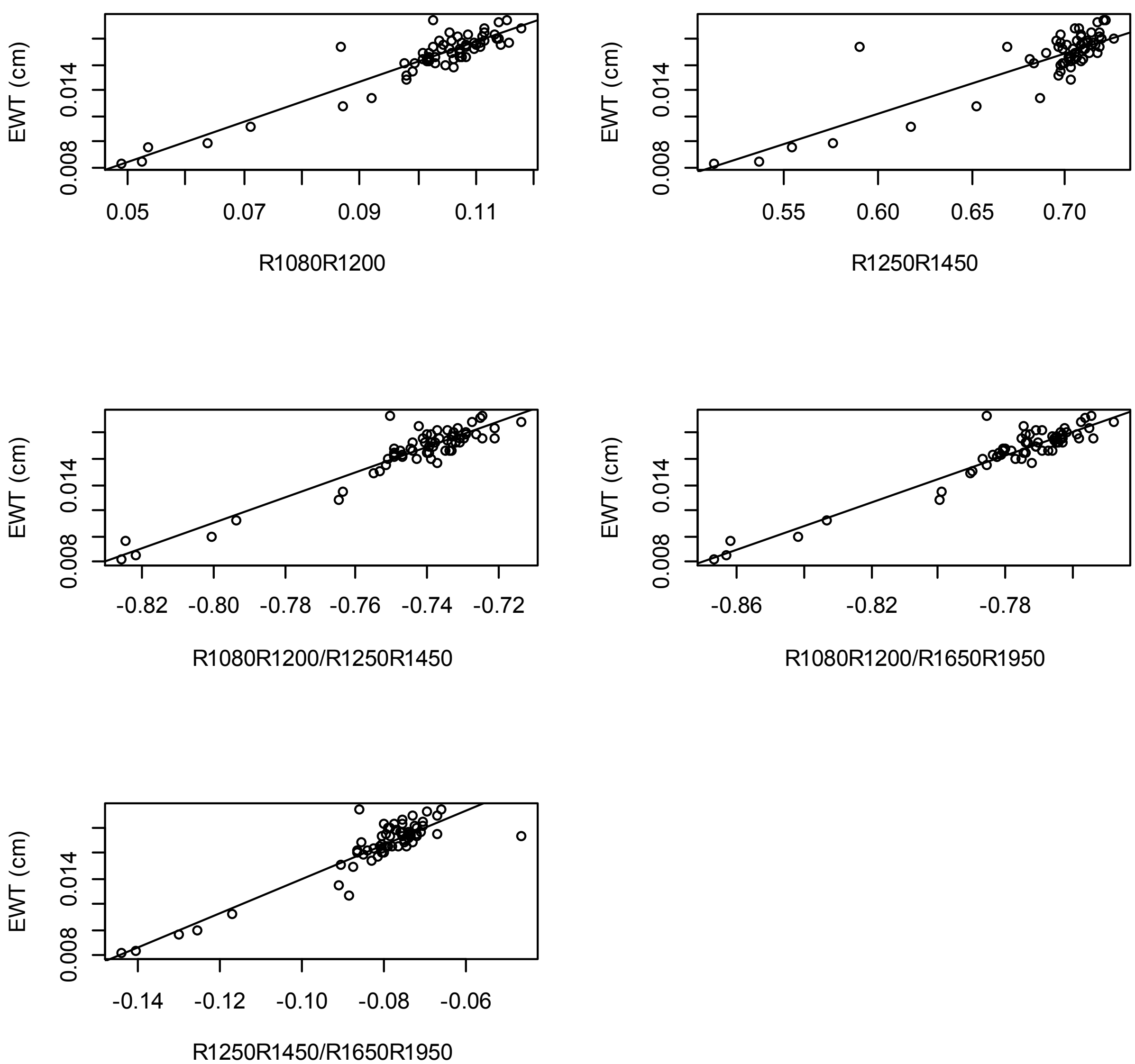


Figure 10. Edited EWT correlations

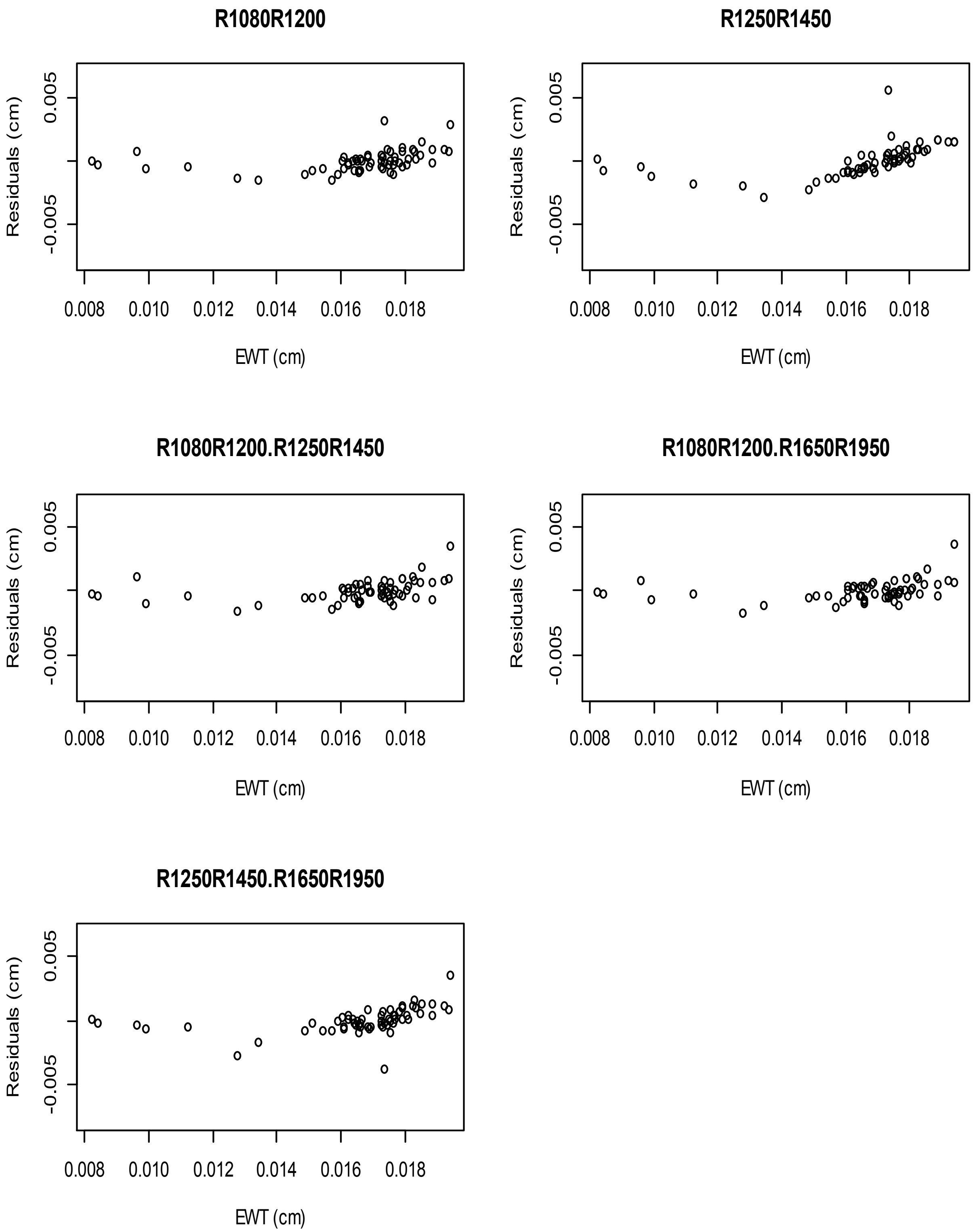


Figure 11. Residuals when peaks method is used in prediction of EWT on the edited data set.

IR spectrometry – partial least squares

PLS analysis makes it easy to add more components to aid in improving the model. It is also ideally suited to the process of analyzing large sets of x values, such as IR spectra. In fact, the analysis of spectra to predict a single y value is one of the common examples, included in the PLS R package used here.

The results of PLS analysis show stronger correlations than those from the above methods. Importantly, the PLS analysis concurs with other literature on this subject; the peaks in PLS coefficient values correspond to the peaks in the leaf scans that are used in other plants. (unedited parity plots in Figure 13, edited parity plots in Figure 14) shows this relationship. The PLS analysis also shows that between 5 and 6 components are sufficient to describe the relationship between water and IR spectrum. As more components are added to the model, the peaks in coefficients become more exaggerated at the same wavelengths as the peaks in IR of the leaf (see Figure 12 below).

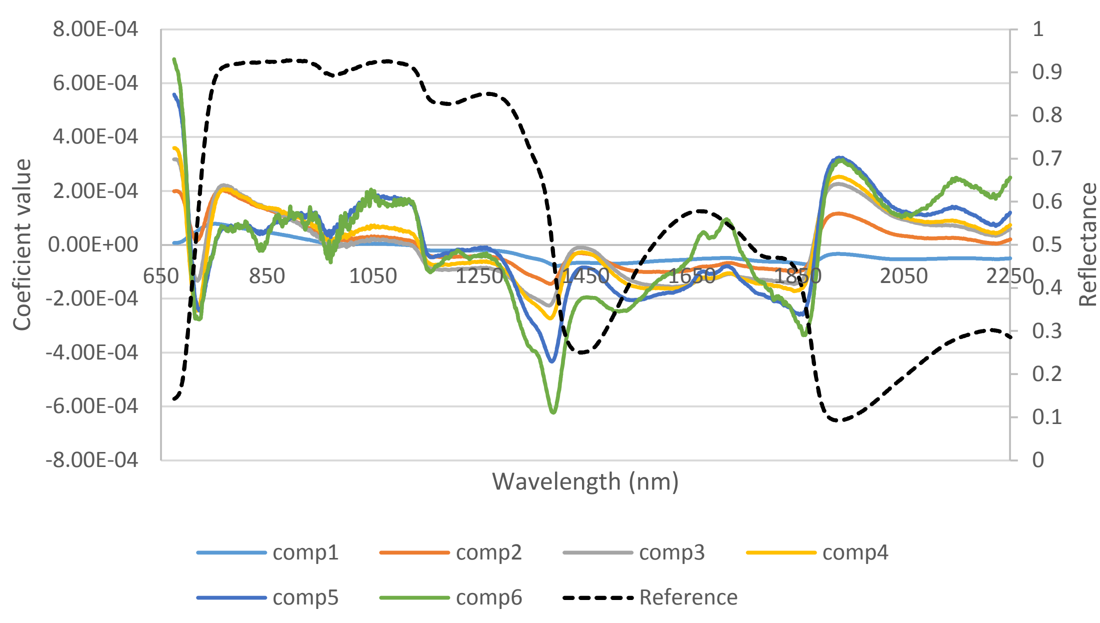


Figure 12. PLS coefficient graph with overlay of reflectance data (reference)

One notable difference between PLS analysis and peak analysis is that removal of the same outliers (edited data set) results in a lower correlation significance when PLS is used than when all data is included, which was not the case for water pressure. The overall R^2 values give high confidence that PLS can be used with IR spectrum to accurately predict the level of water in a leaf.

PLS results comparing water pressure to the IR spectra showed similar results, with peak coefficient values occurring at the peaks occurring in the IR spectrum. However, more components were necessary to get a peak R^2 value (9 components without excluding points for a peak R^2 of 0.72), however, when outliers are removed, the R^2 improves to 0.73 with 7 components.

The PLS method as utilized incorporated took into the entire spectrum when calculating water pressure and EWT. One would expect that this algorithm would result in better results than the previous method. The results of a multi component analysis are shown below in Table 5a, where it can be seen that the peaks and valleys of the coefficients graphs occur at the same points as the peaks and valleys as the IR spectrum. The overall results show that the PLS method when taking into account the entire spectrum yields strong results. Below, in Table 7, the results of the addition of components on the significance of each additional component is tabulated. The best results from this method are as strong as those of the best literature results using other methods such as taking the ratio of wavelengths, and are similar to the second order polynomial found in the other section. An additional effort was made to correlate the y values to the exponential form of the wavelength, in accordance with the data from the peaks method used previously. Since the current data set already has high correlation with EWT, and the water pressure data does not appear to be correlated with the IR spectra through an exponential equation, there is not a dramatic difference in the correlations. The results of the exponential data set are given in 5b.

Table 7. Regression coefficients for data compared to a) unmodified spectra, and b) squared reflectance data

a)

Sample	*1 comp*	*2 comp*	*3 comp*	*4 comp*	*5 comp*	*6 comp*	*7 comp*	*8 comp*	*9 comp*
Unedited Water Pressure	0.32	0.41	0.26	0.44	0.43	0.53	0.62	0.70	0.72
Edited Water pressure	0.62	0.56	0.62	0.66	0.62	0.72	0.74	0.72	0.68
Unedited Water Thickness	0.94	0.95	0.94	0.95	0.95	0.95	0.96	0.95	0.95
Edited Water Thickness	0.79	0.76	0.90	0.90	0.90	0.89	0.89	0.88	0.86

b)

Sample	*1 comp*	*2 comp*	*3 comp*	*4 comp*	*5 comp*	*6 comp*	*7 comp*	*8 comp*	*9 comp*
Unedited Water Pressure	0.38	0.47	0.33	0.56	0.69	0.69	0.72	0.75	0.74
Edited Water pressure	0.39	0.37	0.30	0.31	0.43	0.50	0.58	0.63	0.59
Unedited Water Thickness	0.95	0.96	0.96	0.96	0.94	0.93	0.94	0.93	0.92
Edited Water Thickness	0.85	0.83	0.87	0.92	0.93	0.92	0.92	0.91	0.89

PLS-Water pressure

Table 7 concurs with the individual peaks method that the water pressure method does not generate as high quality of a result as the EWT method. While adding components to the unedited data set increases the significance of the result beyond the number of components tested, the edited data set peaks at 7 components. Examination of the parity plots show that even the edited data set contains approximately 10 bar of uncertainty. The model here consistently underestimates the water pressure. The model especially underestimates the water pressure at higher water deficits. The unedited data set correlations show that as more components are added to the analysis, the correlation increases. There is no end to this trend to the maximum of 9 components used in this analysis. While 9 components were analyzed, the non-smooth lines in the coefficient graph in Figure 12 indicates that the model begins to over fit the data at 6 components.

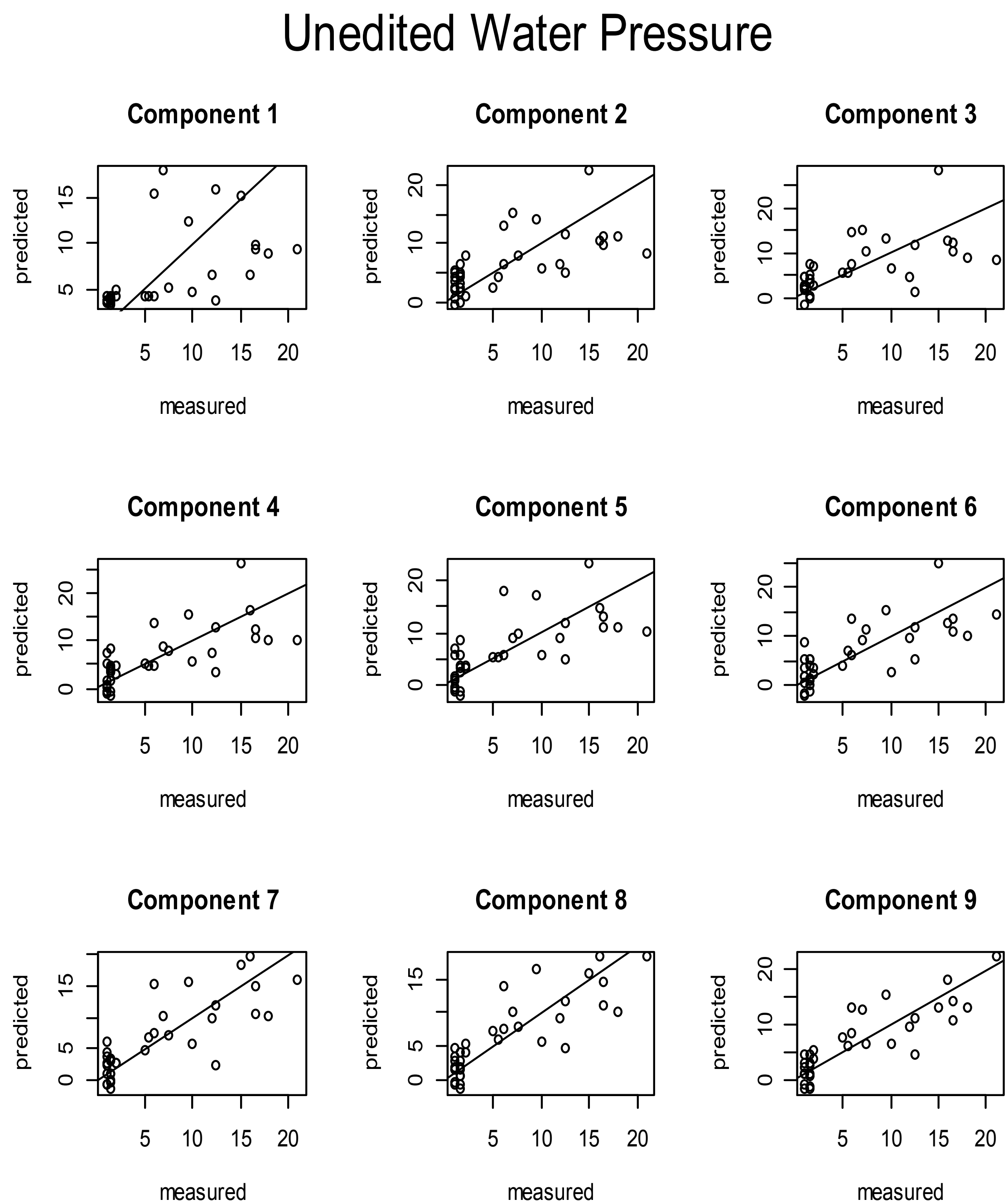


Figure 13. PLS parity plots number of components for the unedited water pressure data set.

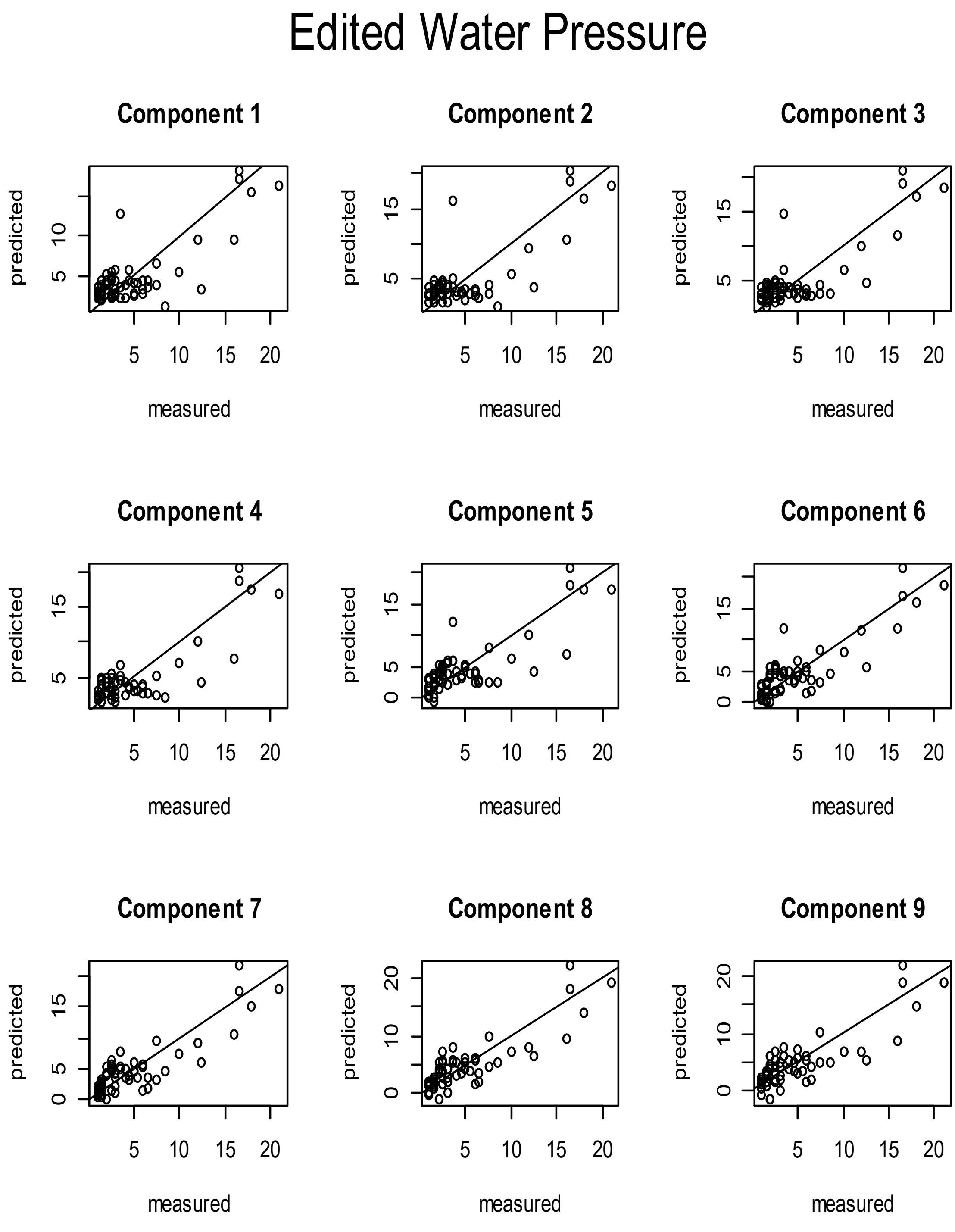


Figure 14. PLS parity plots number of components for the edited water pressure data set.

PLS-EWT

As with the individual peaks method, the results of EWT are significantly better than the water pressure results (unedited parity plots by number of components in Figure 16, edited parity plots in Figure 17). This combination of the PLS and the peaks results shows a greater level of confidence that the low correlation coefficients in the water pressure vs. EWT section was in fact the result of uncertainties in measuring water pressure, or that there are other things in water pressure measurement that make it an indirect measurement of the water content of the leaf.

Also as with the peaks method, the PLS on EWT shows better correlations with the complete data set than with some of the data removed. The parity plots show that there are few outliers when this method is used, and the PLS method results in an overall tight fit. The residuals plots show that the method generates lower overall deviations from the measured results than the linear fit using only peaks.

Just as with the PLS method relating to water stress, the EWT comparison shows that the coefficient values peak and valley where the major spectrum peaks and valleys lie. The plots also show the interesting characteristic of a large peak in coefficient value at the leading edge of the 800 nm peak. It is curious that this peak quickly disappears after the leading edge. These peaks disappear from some of the samples taken, as the lower water content leaves tend to increase in reflectance at higher wavelengths, and less quickly as seen in Figure 15Figure 12. The disappearance of a peak at 800 nm and corresponding slow raise in reflectance between 700 nm and 1000 nm in dry leaves was previously observed by Jacquemoud and Ustin (2008).

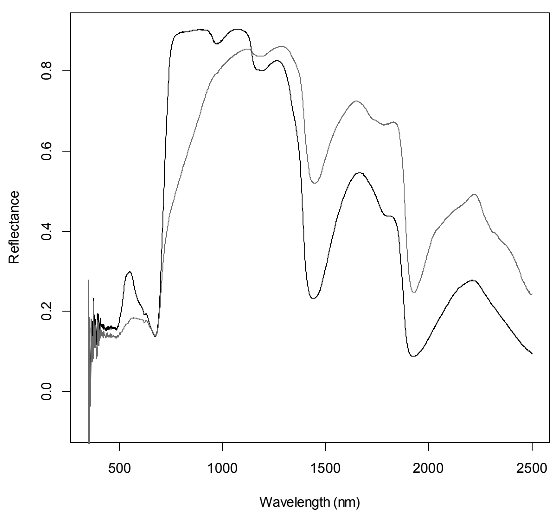


Figure 15. Comparison of two leaf scans. Grey represents a leaf with a slow rise and thus lacking a peak at 800nm. Leaves lacking a peak at 800 nm tend to increase in reflectance at higher wavelengths.

The peak in coefficient values at 1950 nm also agrees with other literature. The reflectance trough at 1950 nm is known to be strongly correlated with water concentration. Given other literature on the subject, as well as the individual peaks methods used above, it is curious that there is no peak in coefficient values at the 1650 nm range, and that the values remain relatively constant

Other papers (Penuelas et al., 1997) have avoided this R_{800} hump altogether. In an effort to characterize the small valley at 970 nm, the group looked at the peak at 900 nm, which they predicted would be clean of interference from other aspects of the leaf, and allow for Beer-Lambert law corrections for leaf thickness. Since this point is closer to the point at 970 nm, it also avoids some of the issues of the later ascent sometimes seen in water deprived plants. Their results focusing on this ratio, however, did not produce a better correlation than those found in this study for 1080 nm and 1450 nm. One of the reasons that this may not have appeared in the peaks analysis s almost no effect on the overall relationships. Meanwhile, the other correlations all involve numbers that was conducted earlier is that while 800 nm is used, it is being divided by a number that ha with high scores on the coefficients charts.

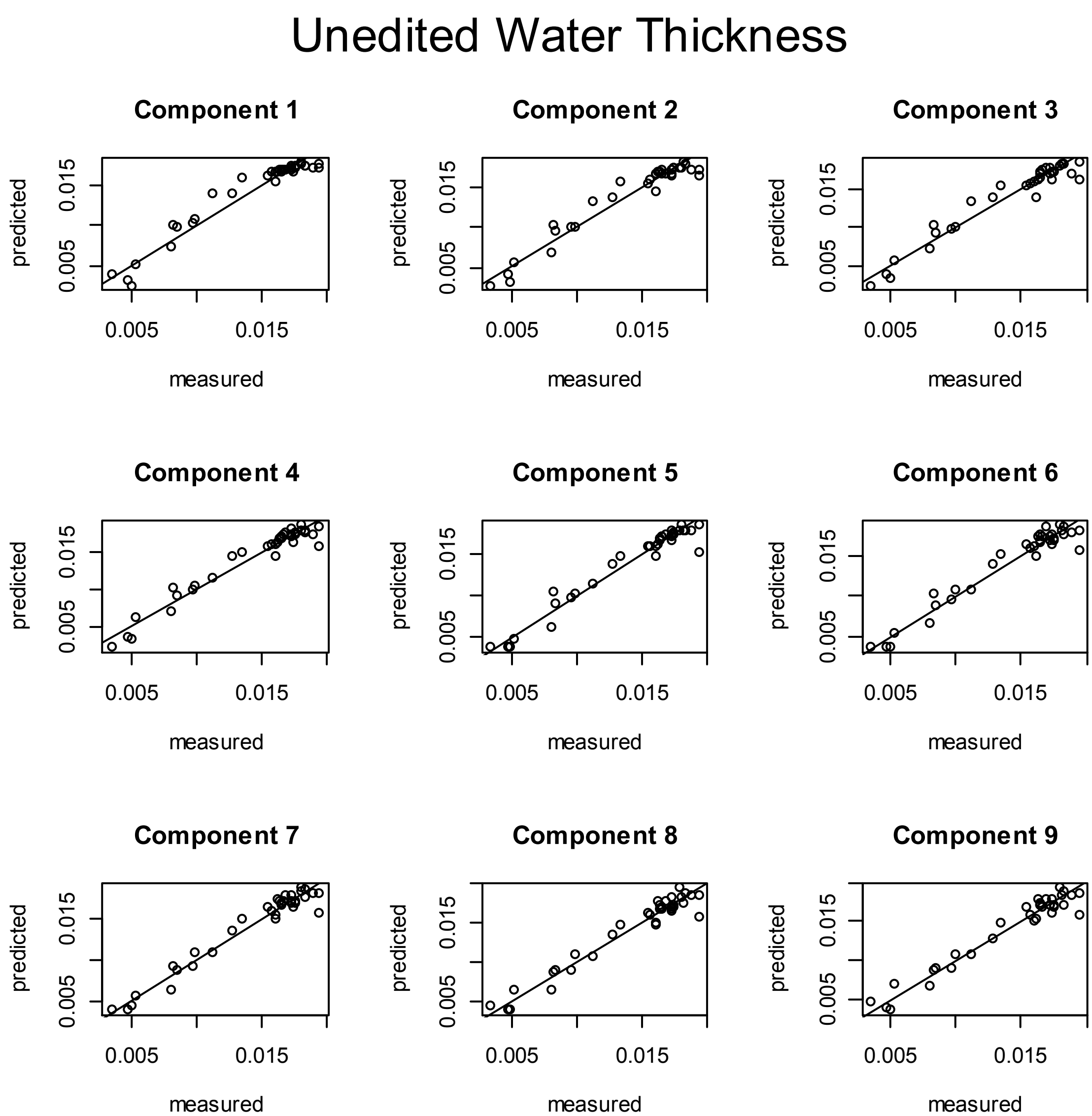


Figure 16. Unedited Water Thickness parity plots for PLS method.

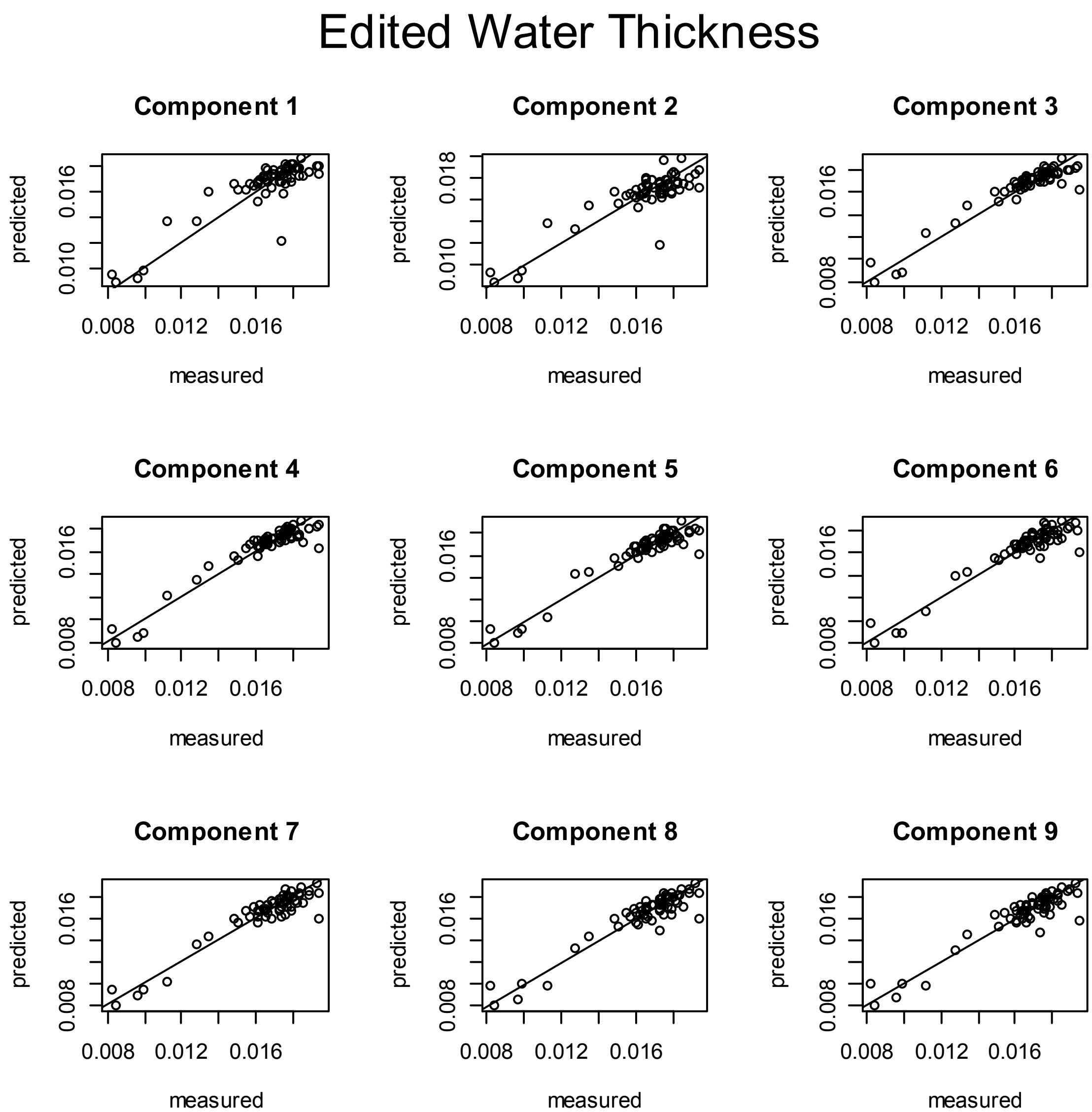


Figure 17. Edited water thickness parity plots for use of PLS.

LED evaluation

The R^2 values are shown below in table 7.

As can be seen from the table, the bands corresponding to 1050nm and 940nm are essentially the same (when examining the graph posted in the previous section, this can be seen clearly as well, provided the 970nm peak exists), meaning that significant savings can be found realized by replacing the 1080nm wavelength with the 940nm wavelength. It is also worth noting the peak R2 value corresponds to the R900/R1300 combination, which, while it did not appear in the other portions of this study, did appear in the studies conducted by (Hunt et al., 1987) None of these values are significantly different from the studies analyzing only discrete values. When the peaks are combined and normalized, there is no significant improvement.

Table 8. Table of R^2 values for combinations of evaluated LEDs.

Wavelength(nm)	**1050**	**1200**	**1300**	**1450**	**1650**
940	0.58	0.72	0.72	0.68	0.68
1050		0.72	0.71	0.67	0.66
1200			0.69	0.65	0.63
1300				0.64	0.60
1450					0.61

In these experiments, the photodiode was assumed to have a relatively even response across the spectrum being examined, although this is not necessarily the case. The overall results of this experiment show that the results from before translate to this type of system smoothly, and suggest the construction of a prototype model to begin testing. The most likely photodiode has an uneven response across these spectra, with roughly linear increase in response as wavelength increases. However, it does incorporate ranges from 800nm to 1700nm and is completely suitable for the best combinations of wavelengths found.

Since the objective of this portion of the experiment was only to determine the potential for generation of a water pressure equivalent, the EWT was not examined, although it is safe to assume, based on the previous work that the same trends hold, only with tighter correlations.

Conclusion

A number of factors were evaluated that are of importance for the construction of a cheap, non-destructive means of measuring leaf water content in coffee leaves (capacitance and infrared spectrometry). When studies are conducted to relate water content to synchronizing the ripening of coffee beans, the leaf water pressure is used, while studies conducted on other plants using capacitance or spectrometry, the effective water thickness is used. After relating these two values, the applicability of the capacitance measurements and infrared spectrometry were evaluated.

A strong relationship between the leaf IR spectra and the EWT was found, similar to those found in the literature, with correlation coefficients as strong as 0.90 when using a normalized index between 1080 and 1200 nm. The overall evaluation of all measurements shows that the measurement of leaf water pressure is less highly correlated with the water content, as well as with the IR spectrum, possibly because other factors in the plant play a role in determining the water pressure measurements.

The capacitance of the leaf, under the conditions of this study, was found to vary too highly to be of use in the construction of a field instrument. The capacitance of leaves mirrored along the central vein of the leaf, but varied highly along the length of the stem. There was no reliable relationship found in this scenario. There is also a strong possibility that the physical structure of coffee leafs are too different from the physical structure of leaves studied by Afzal et al. (Afzal et al.).

The infrared spectrometry, on the other hand, proved to be a useful predictor of the water content of a leaf. The wavelengths used to predict the water content in coffee leaves are similar to those used in many other studies. Two methods were used to relate infrared spectra to water content through two different models. One model was built based on gathering a complete IR spectrum, using PLS regression. The second model built normalized ratios between sets of two wavelengths (peak and trough pairs) to estimate the water content.

Models based on PLS regression gave an R^2 value of 0.95, which is on par or better than the literature when comparing to EWT, but much lower values of 0.75 when relating to water pressure. Partial least squares yielded a less robust model when relating IR spectra to the water pressure. The PLS analysis also showed that the same regions that have been used in the literature, as well as in the other model are the primary regions of interest when measuring water content in coffee. In particular, PLS showed that the 800 nm region of the spectrum should have high predictive properties for leaf water content, whether water pressure or EWT. The PLS analysis also concurred that the regions of 1080 nm and 1200 nm are predictive of the leaf water content, agreeing with the method relating the normalized ratio of 1080 nm and 1200 nm to predict water content.

Models using normalized ratios of wavelengths gave R^2 values as high as 0.9 when relating to EWT, and 0.7 when relating to water pressure. The results suggest that water pressure is related to various indices through a linear correlation, and the EWT is related to IR indices through an exponential correlation.

Overall, it can be concluded that the construction of a cheap, handheld device for the non-destructive measurement of coffee water stress can be easily constructed. Since the device is non-destructive, multiple measurements are easily feasible, allowing for higher levels of confidence. The models constructed from this study largely agree with the literature as it applies to other plants.

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